# COORDINATING CENTER FOR INFECTIOUS DISEASES (CoCID)

**CoCID Genomics Working Group:** In 1996, CDC's National Center for Infectious Disease (NCID) established the Genetic Working Group (GWG) with the purpose of addressing issues concerning the role of genetics in infectious diseases and promoting collaboration between investigators. This working group was further expanded in 2003 to coordinate the genomic research interests of NCID, National Immunization Program (NIP), and National Center for HIV, STD, TB Prevention (NCHSTP) and the Office of Genomics and Disease Prevention (OGDP). The Coordinating Center for Infectious Diseases (CoCID, created May 2004) includes NCID, NIP and NCHSTP.

The overall mission of this working group includes:

a) Identifying and conducting investigations of host genes associated with infectious diseases and vaccine safety that have public health relevance and to which interventions and preventions can be targeted;
b) Building laboratory and epidemiological capacity for genomics;
c) Training public health professionals in laboratory and epidemiological aspects of host genomics and infectious diseases;
d) Communicating information about genomics and infectious diseases to the public through meetings, seminars, web sites, and publications.

## Top Priorities

Seven high priority research areas have been identified for host genetics in CoCID. These reflect areas where CoCID and its partners are making or can make a significant impact in research that leads to new control and prevention methods or development of control and prevention strategies.

1. ***Infectious Diseases:*** resistance and susceptibility to and severity of infectious diseases.
2. ***Chronic disease etiology:*** clarifying host-pathogen interactions in the causation and pathophysiology of chronic diseases such as chronic fatigue syndrome (CFS), arthritis, cancer, and cardiovascular diseases.
3. ***Vaccines:*** response, failure, adverse events; using host genetic information for vaccine design.
4. ***Antimicrobial and adjunctive therapy response:*** failure, adverse events and development of new therapies.
5. ***Prevention:*** identification of genetically at-risk populations for infectious diseases; targeting of prevention strategies to these populations.
6. ***Development of rapid molecular tools*** based on DNA, mRNA and protein expression patterns that can be used in surveillance, studies monitoring response to therapy, and basic-research.

7. ***Drug metabolism:*** defining pharmacogenetic factors associated with response to drug therapy, drug interactions, and disease outcome in tuberculosis and other infectious diseases.

## Major Accomplishments, 2004

A total of sixteen NCID GWG projects were funded internally with annual funding ranging from $30,000 to $60,000 per year from 1998 to 2003. A number of key scientific observations have been published from these studies. In 2004, the GWG hired a Career Development fellow, who has significant experience in human genetics, to support genetic investigations and provide technical guidance on study design and data analysis for investigators in NCID and other centers.

The CoCID GWG conducts cross cutting work to promote host genomics research that includes:

- Collaborations with OGDP in a CDC-wide effort to integrate genomics research into acute public health investigations that involved an expert meeting in May 2004 to formulate a CDC wide strategic plan. This includes developing model consent forms for IRB pre-approval, facilitating storage capability for long-term storage of specimens, technical guidance on study design and methods, technical support for genotyping and data analysis, and outlining criteria to prioritize outbreaks for incorporation of host genomics.
- Ongoing contributions to the CDC-wide effort to use the National Health and Nutrition Survey (NHANES) III DNA Bank to identify the frequency of different mutations in the immunologically relevant host genes and support for data analysis related to infectious disease outcomes. CDC/OGDP has an IRB-approved proposal to generate nationally representative frequency data on variants of genes of public health significance using NHANES III DNA samples. NHANES III is a nationally representative sample of the US population and DNA specimens are available from ~ 7,000 persons that reflect the racial composition of the U.S. population. In 2003, CDC developed a protocol to determine the prevalence of 57 genes of public health significance by looking at 87 different polymorphisms. When complete, this will generate the most comprehensive database of the background frequencies of these genes in a healthy population that is unparalleled in size in the United States. Of the 57 genes chosen for the NHANES project, more than 30 are relevant to infectious diseases. The GWG will assist with the data analysis of infectious diseases related issues. (More detail at: http://www.cdc.gov/genomics/activities/ogdp/2003/chap01.htm).
- Assistance in development and testing of candidate gene selection software tools for genomics research as part of CDC-wide effort.
- Provision of technical and data analysis support for research investigations related to host genomics projects at CoCID.
- Organization and promotion of research and educational seminars in the genomics of infectious diseases.

## Ongoing Initiatives

Important ongoing initiatives are listed by title and Center in Tables 1–3. A description of each listed project is provided in the text that follows, organized by genomics priority research area.

Table 1. **Genomics Research Projects in NCHSTP**

| Title | Division |
|---|---|
| ***Infectious Diseases: Resistance and Susceptibility to Infection and Disease, and Severity of Infectious Diseases*** | |
| • Genomics approach to identify drug targets for a variety of pathogen | DHAP |
| • Influence of host genetics on HIV resistance, disease progression and effect of virological failure on HAART | DHAP |
| • Role of gene polymorphisms in susceptibility to TB in Botswana | DHAP/DTBE |
| • Prospective evaluation of immunogenetic risk factors for susceptibility to TB infection and progression to TB disease | DTBE/DHAP |
| ***Pharmacogenetics of Drug Metabolism and Genetic Risk Factors Associated with Treatment Outcome*** | |
| • N-Acetyl Transferase Type II genotype and TB treatment outcome with isoniazid and rifapentine and rifabutin regimens | DTBE/DHAP |
| • 2C19 polymorphisms and metabolism of nelfinavir | DTBE/DHAP |
| • Evaluation of genetic risk factors for TB treatment outcome | DTBE/DHAP |

Table 2. **Genomics Research Projects in NIP**

| Title | Division |
|---|---|
| ***Genetic risk factors associated with vaccine related adverse outcomes*** | |
| • Establishing registry of clinically significant adverse events and related clinical data and a repository of biological specimens | ESD |
| • Nested case control study of Smallpox vaccination and myo/pericardial injury and inflammation | ESD |
| • Retrospective cohort study to evaluate genetic predisposition to developing rheumatoid arthritis in persons receiving Hepatitis B vaccine | ESD |

Table 3. **Genomics Research Projects in NCID**

| Title | Division |
|---|---|
| ***Integrating genomics into acute public health investigations of infectious*** | |

| | |
|---|---|
| ***diseases*** | |
| • Role of genetic risk factors in Leptospirosis outbreak | DBMD |
| • Outbreak investigations involving Severe Acute Respiratory Syndrome (SARS) | DVRD |
| • Analysis of genetic risk factors for fatal influenza in children | DVRD |
| • Searching for a flavivirus susceptibility or resistance gene in West Nile virus (WNV) outbreak | DVRD |
| ***Infectious Diseases: Resistance and susceptibility to infection and disease and severity of infectious diseases*** | |
| • Genomics research studies in malaria | DPD |
| • Genetic risk factors associated with development of lymphedema in lymphatic filariasis | DPD |
| • Genetic factors in meningococcal and pneumococcal diseases | DBMD |
| • Molecular signatures of cervical neoplasia | DVRD |
| • Integration of gene expression, clinical, and epidemiological data to characterize chronic fatigue syndrome | DVRD |
| • Host gene expression profiles that precipitate post-infective and chronic fatigue syndromes in response to common infections | DVRD |
| • Exercise responsive genes measured in peripheral blood of women with chronic fatigue syndrome and matched control subjects | DVRD |
| • Integration of gene expression and clinical data from CFS andnon-fatigued subjects | DVRD |
| • Development of a text mining tool that provides gene information in specific disease and biological context | DVRD |
| ***Infrastructure*** | |
| • Setting up a high throughput genotyping capacity | SRP |
| • Global amplification of sense RNA: a novel method to archive and replicate RNA for microarrays | DVRD |
| • Beta-test site for emerging genomic technologies | DVRD |
| • Automated tools for genomic research | DVRD/SRP |
| • Development of a gene expression microarray database for flexible data processing, analysis and archiving | DVRD |

**Integrating Genomics into Acute Public Health Investigations of Infectious Diseases**

Host genes play an important role in influencing infectious disease outcomes. Integration of host genomics investigations into outbreak investigations can help identify particular host factors relevant for severe disease outcomes and transmissibility of diseases. Some examples of acute public health investigations involving genomics research have included the following.

- **Role of genetic risk factors in Leptospirosis outbreak** (CDC/NCID/Division of Bacterial and Mycotic Disease (DBMD)/Meningitis and Special Pathogens Branch): The first success story of integrating host genomics into an outbreak investigation at CDC[16]occurred during a leptospirosis outbreak involving triathletes exposed to lake water. HLA-DQ6-positive triathletes had increased risk of laboratory-confirmed leptospirosis (OR=2.8, P=0.04) compared to DQ6-negatives. DQ6-positive triathletes swallowing lake-water had greatest risk (OR 8.46, P< or =0.001). This investigation is the first report of a genetic risk factor affecting susceptibility to leptospirosis and of documented gene-environment interaction (DQ6 and swallowed water) that affected infectious disease susceptibility.

- **Outbreak investigations involving Severe Acute Respiratory Syndrome (SARS)** (NCID/Division of Viral and Rickettsial Diseases (DVRD)/Respiratory and Enteric Viruses Branch)**:** A pilot study to evaluate the role of immunologic and host genetic factors in SARS infection is ongoing using specimens collected as part of the 2003 SARS outbreak.

- **Analysis of genetic risk factors for fatal influenza in children** (CDC/NCID/DVRD/Influenza Branch)**:** A case-control study integrated with multistate population-based surveillance for influenza-related hospitalizations in children during the 2004-2005 season to identify genetic variants that may put certain children at a greater risk of mortality associated with influenza.

- **Searching for a flavivirus susceptibility or resistance gene in West Nile virus (WNV) outbreak** (CDC/NCID/Division of Vector-borne Infectious Diseases (DVBID)/Arboviral Diseases Branch): CDC scientists are currently collaborating with Georgia State University to search for a flavivirus susceptibility/resistance gene in persons who were hospitalized for WNV disease during the 2003 WNV outbreak in Colorado and to study genetic polymorphisms that confer flavivirus resistance in mice, humans and horses with either asymptomatic or overt WNV disease.

**Infectious Diseases: Resistance and Susceptibility to Infection and Disease, and Severity of Infectious Diseases**

A better understanding of the role of various genetic factors in the outcome of infectious diseases will lead to development of novel therapies and better prevention efforts. Recent progress in high throughput genotyping and decoding of the human genome has led to new optimism in identifying such genetic risk factors. The availability of well-

characterized large cohorts from different populations will be critical in identifying such risk factors. CDC has access to epidemiologically well-characterized different infectious disease cohorts. These cohorts are unique resources in identifying such genetic risk factors. Several investigations at CDC that have integrated genomics research are highlighted below.

- **Genomics factors associated with resistance and susceptibility to malaria** (CDC/NCID/Division of Parasitic Diseases (DPD)/Malaria Branch): By taking advantage of a unique community based birth cohort study conducted in Kenya, researchers have demonstrated that sickle cell traits provide significant protection against mortality and morbidity, especially in infancy, before the onset of clinical immunity in areas with intense transmission of malaria[1]. A novel polymorphism in the inducible nitric oxide synthase gene confers protection against severe malaria anemia[13]. In addition, Fc receptor IIA genetic polymorphism was found to be associated with protection against high-density malaria infection in children[30]. This project has led to several collaborative studies involving various academic institutions including the National Institutes of Health (NIH). The ongoing studies focus on identifying the genetic risk factors associated with severe malaria.

  A collaborative study with the Malaria Research Center of India and the Morehouse School of Medicine in Atlanta involves using host genomic approaches to identify potential biomarkers and genetic risk factors associated with cerebral malaria and its associated neurological deficits in collaboration.

  Researchers demonstrated that Fc receptor IIA genetic polymorphism is associated with protection against placental malaria infection in HIV positive women and perinatal HIV infection[5, 6].

- **Genetic risk factors associated with development of lymphedema in lymphatic filarisis** (CDC/NCID/DPD): Using a filarial study of family cohort, investigations are underway to determine the genetic and environmental factors that affect the risk of developing lymphedema due to *Wuchereria bancrofti* infection.

- **Genetic factors associated with meningococcal and pneumococcal diseases** (CDC/NCID/DBMD): Using blood spot specimens collected at birth for state-based newborn screening programs (NBS), efforts are underway to identify genetic risk factors associated with the risk of and susceptibility to severe meningococcal and pneumococal disease, particularly among populations of infants and young children enrolled in routine immunization programs. CDC is collaborating with the Wisconsin EIP (Emerging Infections Program) and NBS program, Wadsworth Lab of New York State University, Emory University, University of Washington, and Molecular Staging.

- **Genomics approach to identify drug targets for a variety of pathogens** (CDC/NCHSTP/Division of HIV/AIDS Prevention (DHAP) and NCID): Using gene

trap technique to identify host cell proteins that are critical for survival of pathogens within host cells, about 200 candidate host genes that potentially play critical roles in the life cycles of Marburg (MBG) virus, Ebola (EBO) virus, HIV-1 and HIV-2, influenza A, or retrovirus have been identified. One candidate gene, Rab9, has been explored as a potential target for anti-viral therapy.

- **Molecular signatures of cervical neoplasia** (CDC//NCID/DVRD): As part of the National Cancer Institute's (NCI) Early Detection Research Network a genomics project to detect and validate biomarkers that can be used to improve the sensitivity and specificity of cervical cancer screening is ongoing. This project focuses on gene expression profiling of exfoliated cervical cells and proteomic profiling of cervical mucous samples using SELDI technology. Protocols were established for optimizing molecular quality of archived biologic samples and the method was published for extracting RNA and DNA from exfoliated cervical cells and for archiving RNA allowing sense global amplification and making cDNA in biorepositories a renewable resource. Candidate biomarkers that were identified are being explored further.

- **The chronic fatigue syndrome (CFS) program to integrate genomics into chronic infectious diseases and illness** (CDC/NCID/DVRD/Viral Exanthems and Herpes Virus Branch (VEHB)): The CFS Molecular Epidemiology Program was established in 1997. The CFS Program was designed to apply rapidly evolving cutting-edge genomics, proteomics, and bioinformatics technology to epidemiologic studies whose objective is CFS prevention and control. Its aim is to characterize CFS at a systems biology level by integrating surveillance, case definition, and clinical studies with genomics, proteomics and bioinformatics. The effort includes data from population-based and clinical studies. Examples of each of these are described below.

    - **Integration of gene expression, clinical, and epidemiological data to characterize CFS**
        - Integrated the peripheral blood gene expression results with epidemiological and clinical data to determine whether CFS is a single or heterogeneous illness
        - Using statistical tests and cluster analysis to distinguish CFS subjects and identify differentially expressed genes
        - The latest results suggest that CFS is a heterogeneous illness. The differentially expressed genes imply fundamental metabolic perturbations that will be further investigated and illustrates the power of microarray technology for furthering our understanding CFS[37]

    - **Host gene expression profiles that precipitate post-infective and chronic fatigue syndromes in response to common viral and rickettsial infections**
        - An example of a CFS gene expression study that is based on model systems

- Studying host gene expression profiles following acute infection with Epstein Barr Virus (EBV), *Coxiella burnetti* (the causative agent of Q fever) and Ross River Virus (RRV) in collaboration with the University of New South Wales in Sydney, Australia**.** Some observations from this longitudinal study include: a) severity of the acute illness is a powerful predictor of the likelihood of development of post-infective fatigue syndrome (PIFS) at three and six months, b) although the pattern and severity of symptoms in the acute illness were correlated with production of pro-inflammatory cytokines, these relationships did not persist through to the PIFS phase of the illness, c) identified several novel gene expression correlates of individual symptoms using microarray gene expression profiling

- **Exercise responsive genes measured in peripheral blood of women with chronic fatigue syndrome and matched control subjects**
  - Measuring peripheral blood gene expression profiles of women with CFS and matched controls before and after exercise challenge to search for markers of CFS-associated post-exertional fatigue, differential expression of exercise-responsive genes classified in chromatin and nucleosome assembly, cytoplasmic vesicles, membrane transport, and G protein-coupled receptor ontologies between CFS patients and controls

- **Integration of gene expression and clinical data from CFS and non-fatigued subjects enrolled in a two day clinical evaluation in Wichita, Kansas**
  - Following a well-characterized cohort of people with CFS over a four-year period to determine if unique gene expression profiles are associated with symptom occurrence or persistence of illness**.** Evaluated each subject and the corresponding multiple samples using a 40,000-gene microarray. Data analysis is in progress. This will also serve as the dataset for $C^3$, the CFS Computational Challenge, (described in section F 'Major Conferences')

- **Development of a text mining tool that provides gene information in specific disease and biological context**

  - Pioneered a number of genomic and bioinformatics technologies at CDC's VEHB (see CDC MAdB in Infrastructure section below)
  - Simultaneous assessment of tens of thousands of genes using high-throughput technology, such as gene expression profiling using microarrays
  - Key to getting specific digital information associated with genes is mining text in the appropriate biological, clinical and epidemiological context. Development of a text-mining tool that will provide biologic

and disease relevant information for genes identified as important by microarray gene expression profiling

- **Influence of host genetics on HIV resistance, disease progression and effect of virological failure on highly active anti-Retroviral therapy (HAART)** (CDC/NCHSTP/DHAP/Laboratory Branch): Researchers have focused on the influence of host genetics on HIV resistance, disease progression, and more recently on the effect of virological failure on HAART therapy in diverse populations. They found for the first time that CCR5 human haplogroup E was associated with an accelerated CD4 count decline to <200 cells/μL in an injecting drug user cohort from Thailand, providing evidence that the CCR5 human haplogroup E accelerates the decline of the CD4 cell count and may lead to accelerated disease progression in an Asian population. Study of the effect of host genetics on virological failure of HAART using a HIV-infected adult cohort with well-documented treatment history is currently undertaken.

- **The role of host genetic polymorphisms in susceptibility to *M. tuberculosis* infection and progression to TB disease** (CDC/NCHSTP/DHAP/Laboratory Branch and Division of TB Elimination (DTBE)):

  Tuberculosis (TB) is the leading infectious cause of death among adults worldwide, with nearly 2 million deaths annually from TB worldwide. Although an estimated one-third of the world's population is infected with *Mycobacterium tuberculosis*, only approximately 10% of infected persons will advance to TB disease, with the remainder maintaining latent infection. The low ratio of disease to infection despite almost universal exposure in certain regions and reports of ethnic differences in susceptibility to disease suggest that host-specific factors may play a major role in progression to TB disease. Identifying genetic risk factors for susceptibility to TB would permit TB programs in other countries to target costly interventions such as contact investigation, treatment for latent TB infection, directly observed therapy, and intensive follow-up towards the 10% of exposed persons truly at risk of developing TB. This targeted approach would result in considerable cost savings, and ultimately, improved TB prevention and control.

  In recent years a role of host genetic polymorphisms in susceptibility to *M. tuberculosis* infection and progression to TB disease has been reported. Based on these reports of genetic associations with susceptibility to TB, polymorphisms in at least 10 genes may influence susceptibility to TB infection and/or progression to TB disease. Despite these single gene associations, no study has reported the role of mutiple genes in a single study. Moreover, all studies to date have been conducted in TB-endemic countries, where multiple TB exposures and use of BCG vaccine limits the possibility of evaluating genetic risk factors for TB infection.

  - **Role of gene polymorphisms in susceptibility to TB in Botswana** (CDC/NCHSTP/ DHAP and NCHSTP/DTBE)

- Investigating the potential role of multiple immune response genes in infection with *M. tuberculosis* and disease progression in a cohort of patients reporting to a respiratory clinic in Gaborone, Botswana in 1998 – 2000
- Assessing the role of polymorphisms in the N-acetyl transferase 2 (NAT2) gene on drug metabolism and treatment failure also in this study
- Significant associations of polymorphisms in the IL-10 gene promoter -819/-592 haplotype and the NOS2 -954 with TB disease and an association of the IFNγ +874 with latent TB infection

- **Prospective evaluation of immunogenetic risk factors for susceptibility to TB infection and progression to TB disease** (CDC/NCHSTP/DTBE, NCHSTP/DHAP, and TB Epidemiologic Studies Consortium)
  - Launched a prospective study of polymorphisms of 17 different candidate genes in an epidemiologically well-characterized population of contacts exposed to infectious tuberculosis patients at 11 sites in the United States and Canada
  - Genotypes under investigation individually and in combination
  - Candidate genes were selected based on literature reports, biologic plausibility, and data from animal models. Genotype and haplotype frequencies for exposed contacts who do and do not develop latent TB infection will be compared. TB registry matches will be conducted annually for 5 years after enrollment is completed to assess progression to TB disease among enrolled contacts. A total of 1300 contacts have already been enrolled into this 5–year prospective study. Enrollment is planned through December 2006

**Pharmacogenetics of Drug Metabolism and Genetic Risk Factors Associated with Treatment Outcome** (CDC/NCHSTP/DTBE, NCHSTP/DHAP, and the TB Trials Consortium)

A series of pharmacokinetic studies undertaken have sought to investigate aspects of the pharmacogenetics of TB therapy. The first efforts involved incorporation of NAT-2 genotypes into the evaluation of isoniazid PK and its relation to treatment failure and relapse. A second effort has sought to assess the impact of possible polymorphisms of a particular cytochrome p450 isoform on the interaction between rifamycins and the anit-HIV protease inhibitor nelfinavir. Additional studies are being launched to evaluate genetic risk factors for TB treatment outcome.

- **N-acetyl transferase type II genotype and TB treatment outcome with once weekly isoniazid and rifapentine and twice-weekly rifabutin regimens**. Emergence of resistance during treatment with once weekly regimens including isoniazid is associated with isoniazid acetylator phenotype, occurring almost exclusively among patients who metabolized isoniazid quickly (so-called “fast

acetylators") and who therefore had a shorter period of drug exposure. Thus, isoniazid acetylator phenotype may be an important factor in the emergence of rifamycin resistance in the rifapentine trial. Isoniazid is metabolized by a polymorphic group of enzymes called N-acetyl transferases. The primary locus involved in INH metabolism is called NAT-2 and methods exist to identify the precise genotypes present in a given individual.

- **2C19 polymorphisms and metabolism of nelfinavir** (DTBE/NCHSTP, DHAP/NCHSTP and the TB Trials Consortium). The P450 isozymes CYP3A4, CYP2C19, CYP2D6 and CYP2C9 are responsible for nelfinavir metabolism *in vitro*. Unlike the other protease inhibitors, which are predominantly metabolized by CYP3A4, approximately one-half of nelfinavir clearance is achieved by CYP2C19. Several minor metabolites and one major oxidative metabolite of nelfinavir are found in plasma. The major oxidative metabolite, NFV hydroxy-t-butylamide (M8), has HIV activity comparable to the parent compound in vitro. The contribution of M8 to the clinical efficacy of nelfinavir is uncertain. Transformation of nelfinavir to M8 appears to occur solely at 2C19, as genetic polymorphism at 2C19 (poor metabolism at CYP2C19 is seen in 3-5% of Caucasians), or drug inhibition of 2C19 resulted in absence of M8 and increased plasma concentrations of nelfinavir.

**Evaluation of genetic risk factors for TB treatment outcome (DTBE/NCHSTP, DHAP/NCHSTP, and the TB Trials Consortium)**. An evaluation of genetic risk factors for TB treatment outcome has been integrated into an ongoing TB clinical trial evaluating nucleic acid amplification tests and epidemiological factors as potential surrogate markers to predict relapse of tuberculosis and to monitor the effectiveness of treatment. Surrogate markers may help to tailor treatment in individual patients and might also permit more rapid and efficient conduct of therapy trials, shortening significantly the total duration of these long and costly studies. The study is co-enrolling patients in TBTC TB treatment trials. Each subject is thoroughly described clinically, radiographically, and by standard TB microbiology. A total of 130 patients have been enrolled to date, out of a planned sample size of 300. The existence of a bank of serial plasma, buffy coat, and sputum specimens from a well-characterized group of patients provides an opportunity also to evaluate possible genetic and serologic markers of treatment outcome. Genotypes to be tested include polymorphisms of MDR1, UGT, Toll-like receptor 2, and IL12B receptor.

**Genetic risk factors associated with vaccine related adverse outcomes (NIP)**

Pre-licensure clinical safety trials and post-licensure signal detection have been the benchmarks to measure vaccine safety. Mainstays of vaccine safety surveillance are the Vaccine Adverse Event Reporting System (VAERS) and the Vaccine Safety Data link (VSD). VAERS is a national passive surveillance system and receives reports of suspected adverse event following receipt of any U.S. licensed vaccine. Its goals are to detect new, unusual, or rare vaccine adverse events; monitor increases in known adverse

events; and determine potential patient risk factors for particular types of adverse events. VAERS was established in 1990 and is jointly administered by CDC and the Food and Drug Administration (FDA). Although VAERS receives approximately 20,000 reports per year, the clinical and laboratory data collected cannot be routinely validated or standardized due to the limitations of a passive surveillance system,

VSD is a collaboration of researchers from private and public sectors who study the safety of vaccines administered to all age groups by conducting epidemiological research using a large linked database. Although, the collective VSD data includes active surveillance of approximately seven million people (2.5% of the total U.S. population), it does not investigate or manage vaccine adverse events on an individual level for the purpose of systematically collecting and evaluating those incidents.

Post-licensure surveillance must be augmented by innovative research that addresses the fundamental pathogenesis of adverse reactions to vaccine. To address these unmet research and surveillance needs, CDC established the Clinical Immunization Safety Assessment (CISA) Network in 2001. CISA unites CDC's expertise with highly regarded national experts in infectious disease, clinical investigation, vaccine studies, and public policy. CISA provides leadership, clinical research experience, and scientific credibility for the novel assessment and monitoring vaccine safety from scientific and clinical perspectives.

A comprehensive vaccine safety surveillance system requires closure of existing research and surveillance gaps that can identify at-risk groups. CISA addresses these gaps through research, which (1) standardizes collection of clinical data; (2) creates a centralized database (registry) to include clinical data, treatment regimes and patient outcomes; and (3) creates of a specimen bank (repository) to store biological specimens from patients who have experienced post-vaccination Adverse Events (AEs). Ongoing efforts include the following.

- **Establishing registry of clinically significant AEs and related clinical data and a repository of biological specimens from patients who have experienced serious post-vaccination AEs.** Adverse events following immunization may be a result of interacting host, pathogen and environmental factors. Because most serious AEs are relatively rare, standardized clinical assessment, treatment recommendations and understanding of the pathogenesis for adverse events following immunization (AEFIs) are lacking.

  The Registry / Repository may be used to guide the development of treatment plans and serve as sources of data and specimens for future research studies designed to test specific hypotheses regarding the causal relationship between AEs and particular vaccines. For example, specimens in the repository may be used for future studies of cytokine responses, pharmacodynamics and the relationship of vaccine strain subtypes, gene expression profiles and gene polymorphisms to AEs from the vaccine under question. Specimens stored in the repository will be linked to related

epidemiologic data (demographic, clinical, exposure history and risk factor). The registry and repository will be valuable resources for future, hypothesis-driven research studies. Such studies will increase our understanding of AEFIs and guide development of re-immunization guidelines for patients who might benefit from further vaccinations but may be at higher risk for AEs.

- **Nested case control study entitled, "Smallpox Vaccination and Myo/pericardial Injury / Inflammation"** in collaboration with CISA network, Department of Defense, University of Washington and National Institutes of Health. Objectives are 1) To determine genetic diversity associated with smallpox vaccination and myopericardial injury and disease (clinically overt or subclinical disease).This will include identifying and evaluating polymorphisms in specific genes that may be associated with risk.identify statistically significant association(s) between risk for clinically overt smallpox vaccine-associated myocarditis and polymorphisms in immune response genes, and 2) To correlate the discovery and understanding of the genetic diversity with the immune response in order to improve the ability to identify risk factors for adverse clinical responses to smallpox vaccine.determine whether this association(s) is also true for subclinical cases.

- **The Vaccine Safety Datalink (VSD) project is currently conducting a retrospective cohort study entitled, "***Is there a genetic predisposition to developing Rheumatoid Arthritis in persons receiving Hepatitis B vaccine*?" This study goal is to determine whether exposure to Hepatitis B vaccine increases the risk of Rheumatoid Arthritis (RA) in adults. Using the automated data from the VSD, clinic visits for health plan members enrolled during the study period are collected. Exposure status is determined from the NCK immunization database and is supplemented by chart reviews and a telephone interview sample. Charts are reviewed for all potential cases to determine case status, intervals between first automated clinic visit for RA and actual onset date as well as exposure status. This VSD adverse events study is still ongoing.

  The RA cases identified are invited to participate in a genetic substudy of the aforementioned VSD study. This substudy seeks to determine if there is a genetic predisposition to developing Rheumatoid Arthritis (RA) following Hepatitis B vaccine (HBV). Consenting patients provide blood specimen and complete a questionnaire on their medical history, exposure status, and demographics. Blood specimens are sent to CDC for HLA typing where mononuclear cells will be isolated and DNA extracted. This genetics study is currently in the data collection phase.

**Infrastructure**

- **Setting up a high throughput genotyping capacity** (CDC/NCID/Scientific Resources Program (SRP)): The mapping of the human genome has led to many changes in the field. This advance has revolutionized the genotyping technology leading to high throughput genotyping as a standard tool for the future. High

throughput genotyping methods are robust, rapid and cost effective. Currently, limited infrastructure and personnel resources for large-scale high throughput genotyping facility exist in CoCID. Hiring the Career Development Fellow for the GWG has facilitated the group's ability to focus on developing infrastructure for high throughput genotyping. This includes exploring ABI SNPlex and MassARRAY™ system from Sequenom for high throughput genotyping.

- **Candidate gene selecting tool and genotyping database:** The CoCID GWG and OGDP are currently developing tools and materials to assist CDC programs in identifying candidate genes and gene pathways to assess the role of genomics in epidemiologic studies and acute public health investigations. The end result of this project will be a suggested approach to identifying candidate genes, criteria for inclusion, pathways of interest, priorities, and an initial list of genes single nucleotide polymorphisms (SNPs). To reach this goal, more focused research and consolidation of data and literature must be performed. To facilitate the needs of large-scale genotyping, efforts on developing genotyping databases across numerous projects are also made.

- **Global amplification of sense RNA: a novel method to archive and replicate RNA for microarrays** (CDC/NCID/DVRD): Biorepositories from population-based epidemiological studies are increasingly important resources for cancer biomarker discovery and validation. The finite amount of mRNA available from each sample and the viable nature of RNA during long-term storage significantly limit the number of studies that can be supported by these priceless collections. Several clever amplification approaches have been developed that allow gene expression profiling to be performed on even the few cells available from microdissection. However, these methods do not address the problem of the viability of RNA during long-term storage, nor the compatibility of the RNA with the wide variety of gene expression profiling platforms and approaches.

  Researchers developed a novel procedure to amplify mRNA into sense RNA (sRNA) [27]. The cDNA intermediate forms a stable biorepository capable of regenerating the complex mRNA profile in the original sample. Because the amplified RNA is in the 5'→3' orientation, it is a synthetic equivalent of mRNA and can be used as the template for any platform or approach to gene expression analysis. The procedure exploits the template-switching activity of reverse transcriptase to incorporate RNA polymerase binding site upstream of single stranded cDNA. sRNA prepared from RNA extracted from human cell lines, tissues, blood and fixed exfoliated cervical cells performed satisfactorily in microarray and real-time RT-PCR assays. sRNA preparation preserved the relative differences in mRNAs spiked at concentrations spanning 5 orders of magnitude (0.00001-0.1%). This reflects the high fidelity of sRNA for mRNA species present at concentrations as low as 0.3 copies/cell. From 40 nanograms of input RNA, one round of sRNA amplification resulted in an approximately million-fold amplification of mRNA, and yielded highly reproducible microarray results (Pearson correlation coefficient 0.97 using MWG 10K arrays and

Resonance Light Scattering technology for signal detection). Global amplification of sRNA should find applications in the RNA archiving of population-based biorepositories for biomarker discovery and validation studies for detection and stratification of cancers and other diseases. This RNA amplification and archiving protocol may also find applications in pathogen discovery projects.

- **Beta-test site for emerging genomic technologies** (NCID/DVRD/VEHB): Viral Exanthems and Herpes Virus Branch served as the beta-test site for two important and emerging genomic technologies. Both technologies aim to improve the reproducibility, sensitivity and specificity of glass microarray hybridizations. In collaboration with Ventana Medical Systems (http://www.ventanamed.com/, Tucson, AZ), VEHB has evaluated a robotic platform for automated microarray hybridization. This automated platform enables simultaneous hybridization of 20 microarrays. Consultations are underway with Ventana Medical Systems on use of the automated hybridization system for high-throughput *in situ* hybridization.

  In collaboration with Genicon Sciences (recently purchased by Invitrogen), VEHB evaluated resonance light scattering gold particles (RLS System) for improved signal detection on microarrays and was the first to demonstrate that as little as 500 ng of total RNA can be labeled with biotin hybridized to glass microarrays and detected with resonance light scattering gold particles resulting in several publications (Ojaniemi H et al, 2003; Habis et al, 2004).

- **Automated tools for genomic** research (CDC/NCID/DVRD and the Scientific Resources Program (SRP)): In 1999, a high-performance microarrayer for spotting biological samples into arrays and a laser-scanner for microarray image detection were purchased. This equipment was transferred to the SRP Core Facility to allow access of custom microarrays to any interested NCID user. Today, the SRP Core Facility produces custom microarrays for many NCID intramural investigators and several extramural investigators (e.g., Emory University).

- **Development of a gene expression microarray database for flexible data processing, analysis and archiving (CDC/NCID/DVRD):** For the past 5 years, VEHB has custom-built a microarray database and analysis package, called CDC MAdB. This custom microarray database allows integration of microarray gene expression data with epidemiologic and clinical datasets (non-gene data). Large-scale analysis of gene expression data obtained from microarrays is at the cutting edge of biomedical research. There are many commercial and academic microarray analysis packages available. Most are designed for analysis of specific microarray formats and none have integrated epidemiologic and clinical data. Because of this shortcoming, CDC developed a MAdB to serve as a data warehouse and also a flexible and powerful front-end microarray data processing tool. Because of CDC MAdB's flexible and modular design, it is amenable to integration of non-gene data. Integration of epidemiologic and clinical common data elements with microarray expression data will allow for selection, grouping and/or stratification of microarray

data based on variables deemed to be important risk factors of the illness. Data integration will also allow for exploration of clinical and epidemiologic variables that may have a novel affect on gene expression. Operationalization of this integration for one disease will provide the model integration template for other diseases. CDC MAdB was designed to allow for global microarray data exchange and sharing and is available to all CDC microarray users.

**Training**

- **Leadership Management Institute Training:** CoCID GWG members have been selected to receive CDC's Leadership and Management Institute training (2004-2005). This program will provide training for improving team effort, nurture leadership qualities, and promote collaborative spirit. As part of this training, members are working on developing a strategic plan for CoCID to integrate genomics research for public health application.

- **Consultation:** Provided consultation for various investigators on study design issues, methodological aspects of genotyping consent form development, and data analysis.

- **Seminars and journal club:** Organized select seminars and journal clubs to highlight significant new developments in this field.

**Major conferences**

- **Integrating Disparate Data to Simulate Lymphocyte Function (CDC/NCID/DVRD):** The CDC CFS Research Program sponsored a workshop, *Integrating Disparate Data to Simulate Lymphocyte Function*, at the Banbury Center, Cold Spring Harbor Laboratory, on September 19-22, 2004. The objective was to discuss current knowledge concerning lymphocyte function and to identify means by which computational modeling could be used to understand how this complex biologic system functions in persons with CFS. The workshop brought together experts in immunology, molecular biology, computer sciences, and molecular modeling. Specific aims were to 1) define the types of laboratory and clinical data involved in the current concept of lymphocyte function in normal and abnormal states; 2) present approaches for integrating genomic, proteomic, clinical, and epidemiologic data in such models; and 3) define the level of abstraction and types of assumptions necessary to create the next generation of molecular models.

- **$C^3$: The CFS Computational Challenge:** The CFS Research Program is hosting a CFS Computational Challenge (C3). The results of this challenge will help elucidate the pathophysiology of CFS, identify markers of CFS (or subsets of CFS) that may be useful for effective diagnosis and treatment of CFS, and formulate hypotheses to test in future studies.

The CFS Research Program has conducted a 2-day in-patient clinical study of 227 persons identified with CFS, other unexplained chronically fatiguing illnesses, and randomly selected non-fatigued controls from the general population of Wichita, Kansas. Subjects were carefully evaluated medically and psychiatrically. Investigators obtained measurements of their neuroendocrine status, cytokine profiles, sleep, cognitive function, and evaluated their lifetime stress history and coping mechanisms. To classify parameters of CFS, they evaluated disability, fatigue characteristics, and the impact of cumulative symptoms. Finally they measured expression levels of 40,000 genes in peripheral blood cells.

The challenge will engage computer scientists, bioinformaticians, statisticians, biologists and clinicians to mine biologically and clinically meaningful information relevant to diagnosis and therapeutic intervention of CFS from the Wichita Clinical Study data set. Participants will be organized into teams. The challenge will begin with a 1-day workshop where an introduction to CFS will be given along with a description of the dataset for $C^3$. Each teams results will be presented as a paper and judged for biological and mathematical soundness by an expert panel. All participants will present their results at the Banbury Center, Cold Spring Harbor Laboratory, September 18-21, 2005.

- **CoCID GWG contributed to and attended CDC sponsored conference**: *Incorporating Genomics into the Acute Public Health Response*. Atlanta, GA - May 2004.

## Future directions

CDC is in a unique position to integrate and advance host genomics research by virtue of its access to valuable population based cohorts, outbreak investigations, the repository of specimens associated with vaccine adverse events, and expertise of epidemiology and laboratory science. Although the concept of targeting genetically at risk populations for appropriate interventions to improve their health is not new (e.g.: sickle cell patients owing to mutations in the hemoglobin gene are targeted for antibiotic propohylaxis and this has led to improved life span for these patients), recent advances in genomics research provides novel tools for identifying genetically at risk populations for various risks of infectious diseases and vaccine adverse events. Thus, CDC can promote the research that will help to identify genetic risk factors associated with various outcomes of infectious diseases and to gather knowledge from the published studies to identify genetically at risk individuals for better prevention efforts. In addition, this field is likely to open up ways to identify genetically at risk individuals for vaccine adverse events and therapeutic failure especially for chronic infectious diseases such as AIDS and TB. CDC will have to develop ways to integrate these developments for prevention efforts. In order to accomplish this goal increased efforts are needed in the following areas.

- Resources: Laboratory infrastructure not only to conduct intramural genomics research but also establishing core competencies in evaluating reliable technologies for accurate genotyping of U.S. population. Dedicated funding will be required to support genomics research. Awards by competitive selection for the best proposals for integrating new genomics research of programmatic relevance into ongoing epidemiologic projects from investigators within the three CoCID Centers. Awards are planned annually, and will fund specimen collection and testing costs associated with adding a genomics component to ongoing epidemiologic field studies.
- Personnel: Rapid development in genomics research has led to novel and complicated laboratory methods and data analysis. In addition, various bioinformatics tools have become available for data analysis and information extraction. It is important to bring appropriately trained individuals in these areas to the CDC work force. Training in genetic epidemiology will also be relevant in the future.
- Training: CDC epidemiologists and laboratory scientists will benefit from dedicated training funds to update their laboratory training in new areas and to participate in scientific meetings to increase their awareness of latest development in this field. Research Fellowships can also be helpful to bring in young talent interested in this area of research.

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